REMARKS

Information Disclosure Statement (IDS)

The Examiner requests that Applicants point out the subject matter that is closely related to the present application of the co-pending applications cited on page 2 of the transmittal letter of the Supplemental IDS filed September 6, 2000.

Applicants cited the co-pending applications because they contain a reference to WAP and/or MMTV. However, Applicants believe that the claimed subject matter of the instant application is patentably distinct from the teachings in the cited co-pending applications. The reference to WAP or MMTV can be found in the cited co-pending applications as follows: 1) 08/808,827, page 11; 2) 08/925,214, page 13; 3) 08/999,690, page 14; 4) 08/996,460, page 15; 5) 09/058,546, page 14; and 4) 09/160,0667 and 09/442, 979, page 14.

Rejection of Claims 4, 5, 9, 14, 29, 30, 36, 44, 45, 47-50, 55-57, 62, 76 and 84 under 35 U.S.C. §112, second paragraph

Claims 4, 5, 9, 14, 29, 30, 36, 44, 45, 47-50, 55-57, 62, 76 and 84 are rejected under 35 U.S.C. §112, second paragraph "as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention" (Office Action, page 2).

The Examiner states that the phrase "U3 region" in Claims 4, 9, 14, 29, 36, 57, 62, 76 and 84 and the phrase "0.6 Kb PstI murine MMTV promoter fragment" in Claims 5 and 30 render the claims vague and indefinite" (Office Action, pages 2 and 3).

Applicants respectfully disagree. MMTV is a well known retrovirus and its genomic organization is also well known. Characterizing genomes using restriction enzyme mapping is a well established methodology in the art. Consequently, Applicants' description of a 0.6 Kb PstI promoter fragment present in the U3 region of the MMTV promoter (see, for example, Examples 2 and 4 and Figures 4 and 5 of the subject application) is perfectly clear to the person of skill in the art.

The Examiner states that the phrase "proximal 445 bp of the murine WAP promoter in Claims 44 and 55 and the phrase "320 bp XhoI/XbaI of the murine WAP promoter" are vague and render the claims indefinite.

Applicants respectfully disagree. The WAP promoter is also well known in the art. Furthermore, Applicants clearly teach in the specification as filed that "the region of the WAP promoter which is required for mediating the mammary gland specificity is a 320 bp XhoI\XbaI restriction fragment (-413 to -93)" (specification, page 11, lines 17-19).

The Examiner states that Claims 47-50 are indefinite because they depend from canceled Claim 46.

Claim 47 has been amended to depend from Claim 41 with proper antecedent basis.

The Examiner states that the phrase "rodent MMTV" in Claims 74-81, 91 and 92 renders the claims indefinite because MMTV is the abbreviation for mouse mammary tumor virus.

The claims have been amended to delete the term "rodent".

Rejection of Claims 1, 2, 4, 5, 9-14, 16-19, 23-33 and 36-94 under 35 U.S.C. §112, first paragraph

The rejection of Claims 1, 2, 4, 5, 9-14, 16-19, 23-33 and 36-94 is maintained. The Examiner states that the "claims read on expression of a therapeutic gene *in vitro* or *in vivo*" (Office Action, page 5). The Examiner states that the "expression of a β-galactosidase in explanted normal primary human mammary tissue with vectors pMMTV-BAG and pWAP-BAG is not considered to enable therapeutic gene expression under the control of a MMTV promoter or a WAP promoter, since expression of a marker gene does not correlate with expression of a gene *in vivo*, such that the expression provides for a therapy" (Office Action, page 6).

Applicants respectfully disagree. The test of enablement is whether one of skill in the art could make or use the invention from the disclosures in the patent coupled with information known in the art without undue experimentation (*United States v. Telectronics*, 8 U.S.P.Q.2d 1217, 1233)). Using routine experimentation and the guidance Applicants provide in the specification, a person of skill in the art can make Applicants' claimed vector which comprises a heterologous gene under transcriptional control of an MMTV or WAP regulatory sequence, using a heterologous gene other than those described in the specification (*i.e.*, the β-gal gene or the

cytochrome P450 gene). Furthermore, using routine experimentation and the guidance Applicants provide in the specification, one of skill in the art can use Applicants' claimed vectors to express the heterologous gene *in vitro* or *in vivo*.

Applicants' invention is based on the surprising discovery that "the WAP and MMTV regulatory regions are able to direct expression of a linked heterologous gene in primary human mammary cells, including human mammary carcinoma cells" (specification, page 11, lines 2-6), since the WAP gene "has no human homologue" (specification, page 2, lines 20-21) and the MMTV regulatory region is of murine origin. In the specification as filed, Applicants show how to make a retroviral construct in which β-gal is placed under the transcriptional control of a WAP or a MMTV regulatory region (Examples 1-3); how to make retroviral particles produced by culturing a packaging cell line harboring the retroviral vector and one or more constructs coding for proteins required for the retroviral vector to be packaged (Example 4); andhow to infect mammary cells using supernatant containing the retroviral particles *in vitro* (Example 4). Applicants also show methods for assessing whether the WAP or MMTV-U3 regulatory sequence drives expression of a heterologous gene within a vector in human cells, such as primary human mammary gland cells (specification, pages 30-31). Applicants further describe how to make a retroviral vector carrying the cytochrome P450 gene, which catalyses the hydroxylation of the commonly used cancer prodrugs CPA and ifosfamide to their active toxic forms, under control of the WAP regulatory sequence; how to encapsulate a packaging cell line containing the claimed construct and how to implant the capsules which produce viral particles in or around mammary tissue to ensure continuous release of virus *in vivo* (Example 5).

The Examiner notes states that "[t]he quantity of experimentation required to practice the invention as claimed *would include isolation of any therapeutic gene which is yet to be identified*" and "*determination of the function of said therapeutic gene*" (Office Action, page 11, emphasis added).

Why does the person of skill in the art have to use a therapeutic gene yet to be identified and determine its function? A person of skill in the art can select from a variety of therapeutic genes, for which identifying information such as sequence and function, were known at the time of Applicants' invention. A therapeutic gene discovered subsequent to Applicants' invention can

also be used in the claimed vector, however, the practice of Applicants' invention does not require the discovery of a new therapeutic gene and the determination of its function.

The Examiner further states that the quantity of experimentation required to practice the invention as claimed includes "generation of a DNA construct or a recombinant retroviral vector comprising a heterologous gene under transcriptional control of a MMTV or a WAP regulatory sequence, generation of a retroviral particle or cells containing said DNA construct or retroviral vector, generation of encapsulated cells comprising a core containing said cells, determination of therapeutic effects of the DNA construct, retroviral vector and encapsulated cells on treating disease or disorder, or disorders or diseases of human mammary cells including human mammary carcinoma *in vivo*" (Office Action, page 11).

However, that type of experimentation is well known to those of skill in the art. Using Applicants' guidance provided in the specification and routine skills, a person of skill in the art can substitute a known heterologous gene for the β-gal or cytochrome P450 gene described in the specification as filed, without undue experimentation. A person of skill in the art can make the constructs and the encapsulated cells, administer such constructs or encapsulated cells *in vivo*, and determine any therapeutic effects of the expressed heterologous gene.

It is the Examiner's opinion that the "specification fails to provide adequate guidance and evidence that administration of a vector expressing a β-gal or any therapeutic gene product *in vitro* or *in vivo* would provide sufficient expression of said β-gal or said therapeutic gene product for a duration of sufficient time to effect therapeutic effects for a particular disease or disorder, such as disorders or diseases of human mammary cells *in vitro* or *in vivo*" (Office Action, page 7).

Applicants respectfully disagree. β-gal is typically used by those of skill in the art to establish the effectiveness of a particular protocol or therapy. As pointed out in the previously filed Amendment B, Chen *et al.* (Exhibit A) showed that a recombinant adenovirus which expressed β-gal could be used to treat the β-gal-expressing adenocarcinoma. Chen *et al.* note that the β-gal model "is an artificial one" (Chen *et al.*, page 230, column 2), however, as the Examiner points out, *based on their β-gal data, Chen et al. teach "the use of recombinant adenovirus for the treatment of human cancers* but does not specifically conclude or suggest the role of β-gal in the treatment of any cancer" (Office Action, page 14, emphasis added). The

Chen *et al.* reference is clear evidence that those of skill in the art would recognize and reasonably conclude that Applicants' *in vitro* β-gal model correlates to the use of Applicants' claimed constructs *in vivo*, for example, to treat human mammary carcinoma (see previously filed Amendment A, page 14, the discussion of *In reBrana*, 34 U.S.P.Q.2d 1436,1441 (Fed, Cir. 1995)).

Applicants maintain that the Examiner has failed to meet the initial burden of providing acceptable evidence or reasoning as to why the truth or accuracy of Applicants' statement in their supporting specification is doubted (In re Marzocchi & Horton 169 U.S.P.Q. 367, 369 (CCPA 1971)). In addition to the Orkin *et al.*, Shao *et al.* and Aebischer *et al.* references, the Examiner cites the Verma *et al.* and Eck *et al.*, Petitclerc *et al.* references in support of the enablement rejection.

The Orkin *et al.*, Shao *et al.* and Aebischer *et al.* references have been discussed in the previously filed Amendment A and Amendment B. As with the Orkin *et al.*, Shao *et al.* and Aebischer *et al.* references, the newly cited art does not provide acceptable evidence or reasoning that the truth or accuracy of Applicants' statement in their supporting specification should be doubted.

Verma *et al.* review delivery vehicles used for gene therapy protocols and discuss the obstacles that have emerged. Like Orkin *et al.*, Verma *et al.* discuss the lack of success stories in *clinical trials*. As pointed out in the previously filed Amendment B, the court has clearly stated that:

> There is nothing in the patent statute . . . which gives the Patent Office the right or the duty to require an applicant to prove what compounds or other materials which he is claiming, and which he has stated are useful "pharmaceutical applications," are safe, effective, and reliable for use with humans. It is not for us or the Patent Office to legislate and if the Congress desires to give this responsibility to the Patent Office, it should do so by statute (*In re Krimmel* 130 U.S.P.Q. 215, 220 (CCPA 1961)).

Nevertheless, Verma *et al.* clearly teach the successful use of vectors in gene therapy. For example, Verma *et al.* teach that for short term expression of a gene, "ideal vectors", such as the adenoviral vector exist (Verma *et al.*, page 241, Table entitled "What makes an ideal vector").

Similarly, Eck *et al.* review the "therapeutic issues and current strategies being explored that apply gene therapy to...[a] wide range of diseases" (Eck *et al.*, abstract). Like Verma *et al.*,

Eck *et al.* point out obstacles to gene therapy, however, Eck *et al.* discuss the successes seen with gene therapy protocols. For example, Eck *et al.* teach that retroviral vectors "have the greatest clinical use so far" although their application "is limited to dividing cells" (such as cancer cells) (Eck *et al.*, page 83); that clinical trials using adenovirus, although limited to cystic fibrosis, are ongoing (Eck *et al.* page 86); that administration of purified uncomplexed plasmid DNA "has been shown to be highly effective" in skeletal and cardiac muscle (Eck *et al.*, page 90); that DNA-coated gold particles can be used to deliver genes into superficial cells of the skin or into skin tumors, and although gene expression lasts only a few days, it is likely a function of the targeted cells (e.g., skin cells that are sloughed), rather than a function of the delivery vehicle (Eck *et al.* page 90); and that liposome-mediated transfection "offers a nontoxic, nonimmunogenic means to deliver DNA to a variety of tissues" (Eck *et al.*, page 91).

The Examiner cites Petitclerc *et al.* as teaching that "rabbit WAP promoter and MMTV LTR are highly efficient in directing gene expression *in vitro* in various cell lines but they are only moderate efficient in transgenic animals" (Office Action, page 12). The Examiner concludes that "although the MMTV promoter or Wap promoter could direct gene expression *in vitro*, it would be unclear whether both MMTV promoter and WAP promoter could direct gene expression *in vivo* after gene transfer of the desired gene into a subject in vivo for gene therapy" (Office Action, page 12).

Applicants respectfully disagree. Petitclerc *et al.* clearly teach that expression of a heterologous gene under the transcriptional control of the rabbit WAP and MMTV in mammalian *in vitro* and indicates that expression of the heterologous gene will occur *in vivo*, albeit not as efficiently. Such would be expected since *in vitro* expression occurs in a more controlled laboratory setting.

Applicants maintain that the Examiner has failed to meet the initial burden of providing acceptable evidence or reasoning as to why the truth or accuracy of Applicants' statement in their supporting specification is doubted (In re Marzocchi & Horton 169 U.S.P.Q. 367, 369 (CCPA 1971)). Rather, the cited art provides evidence that those of skill in would reasonably conclude that Applicants claimed vectors and constructs could be used *in vivo* to, for example, express the heterologous gene in a cell or as a pharmaceutical composition. Indeed, the Chen *et al* reference of record is evidence that those of skill in the art would recognize and reasonably conclude that

Applicants' *in vitro* β-gal model correlates to the use of Applicants' claimed constructs *in vivo*, for example, to treat human mammary carcinoma

Applicants have clearly provided an enabling disclosure for the full scope of the claimed invention.

Rejection of Claims 1, 2, 9, 12-14, 16, 17, 26, 27 and 36 under 35 U.S.C. §102(a)

Claims 1, 2, 9, 12-14, 16, 17, 26, 27 and 36 are rejected under 35 U.S.C. §102(a) "as being clearly anticipated by Gunzburg et al., WO 96/07748" (Office Action, page 16).

The Gunzburg *et al.* application has a publication date (March 14, 1996) which is after Applicants' priority date (September 6, 1995). In the subject application, Applicants claim priority to PCT/EP96/03922, filed September 6, 1996, and DK 0976/95, filed September 6, 1995. Applicants perfected their claim to priority by filing certified copies of PCT/EP96/03922 and DK 0976/95 (both of which are in the English language) with the previously filed Amendment A.

Since Applicants' foreign priority filing date antedates the publication date of Gunzburg *et al.* and Applicants' claim to priority has been perfected, the rejection under 35 U.S.C. §102(a) has been overcome 9see MPEP 201.15, 706.02(b)).

Rejection of Claims 1, 2,, 4, 5, 9-14, 16-19, 23-33, 36, 74-81, 91 and 92 under 35 U.S.C. §103(a)

Claims 1, 2, 4, 5, 9-14, 16-19, 23-33, 36, 74-81, 91 and 92 are rejected under 35 U.S.C. §103(a) "as being unpatentable over Dranoff et al., 1993 (U2) in view of Lefebvre et al., 1991 (V2), Wilson et al., 1995 (X3), Archer et al., 1994 (U4), Gunzburg et al. WO 96/07748 (IDS-AQ) and Shao et al., 1994 (X2)" (Office Action, page 18). The Examiner states that Dranoff *et al.* teach subcloning DNA sequences encoding cytokines and adhesion molecules into the retroviral vector MFG which contains the Mo-MuLV LTR and introducing the resulting construct into CRIP packaging cells to generate recombinant virus which are used to transfect B16 melanoma cells, which are inoculated into C57BL/6 mice to monitor the delay of tumor formation associated with the synthesis of cytokine transgene. The Examiner states that Dranoff *et al.* do not teach "using MMTV promoter for the expression of a gene in a retroviral vector in human cells, and a capsule encapsulating the packaging cell line and said capsule comprising a porous capsule wall surrounding said packaging cell line" (Office Action, pages 18-19). The

Examiner cites Lefebvre *et al.* as revealing the presence of MMTV promoter and the positive and negative regulatory regions upstream of the MMTV promoter. The Examiner cites Wilson *et al.* as teaching "transfection of human corneal endothelial cells with pMTV-D305 plasmid vector containing SV40 large T antigen (LTAg) under the control of mouse mammary tumor virus (MMTV) promoter and shows that SV40 LTAg mRNA continued to synthesize at significant levels in pMTV-D305-transfected cells in the absence of the inducer dexamethasone" (Office Action, page 19). The Examiner cites Archer *et al.* as teaching "transfection of the human mammary carcinoma-derived cell line T47D(A1-2) with plasmid expressing luciferase reporter gene under control of MMTV promoter and shows the luciferase gene is highly inducible by either glucocorticoids or progestins" (Office Action, page 19). The Examiner cites Gunzburg *et al.* as teaching "construction of a retroviral vector undergoing MMTV promoter conversion" (Office Action, page 19). The Examiner cites Shao *et al.* as teaching microcapsules composed of collagen and encapsulated B16-F10 cells transduced with retrovirus containing GM-CSF gene into said microcapsule, and the monitoring of GM-CSF secretion in the culture medium.

It is the Examiner's opinion that:

> [i]t would have been obvious to a person of ordinary skill in the art at the time the invention was made to substitute the Mo-MuLV LTR with MMTV promoter and use with any desired gene for the construction of a recombinant retroviral vector, a recombinant retrovirus containing said retroviral vector or packaging cells harboring said retroviral vector, and a capsule encapsulating said packaging cells for the expression of any desired gene product in mammary cells *in vitro* or *in vivo*, because Mo-MuLV LTR and MMTV promoter both are regulatory sequences derived from LTR and they both have function of directing gene expression and both Wilson and Archer teach gene expression under control of MMTV promoter in human mammary carcinoma cells and human corneal endothelial cells (Office Action, page 20).

The Examiner further states that one having ordinary skill in the art at the time the invention was made would have been motivated to do so in order to produce a retroviral vector, a recombinant retroviral particle, a retroviral provirus, a packaging cell line and a capsule using the retroviral vector as taught by Dranoff *et al.* and Shao *et al.* "for generating a potent, specific and long lasting anti-mammary tumor immunity as taught by Dranoff and Lefebvre or to study the modulation of the chromatin environment by steroid receptors in defining their capacity to

regulate gene expression and the mechanisms that regulates the proliferation of the human corneal endothelial cells as taught by Archer and Wilson" (Office Action, pages 20-21).

Applicants respectfully disagree. As amended, Applicants' claimed invention relates to a retroviral vector or a DNA construct comprising a heterologous gene placed under transcriptional control of a 0.6 Kb PstI MMTV promoter fragment, wherein the MMTV promoter fragment directs expression of the heterologous gene in a cell when the vector is introduced into the cell.

As pointed out above, the Gunzburg *et al.* reference was published after Applicants' priority date, and thus, is not prior art to Applicants' claimed invention. The teachings of Dranoff *et al.*, Lefebvre *et al.* and Shao *et al.* have been discussed in the previously filed Amendments A and B. As pointed out in Amendments A and B, Dranoff *et al.* and Shao *et al.* do not even mention the MMTV promoter or the use thereof for any purpose. Thus, the teachings in the Dranoff *et al.* and Shao *et al.* references are not relevant to Applicants' invention.

Lefebvre *et al.* identified two regions of the MMTV LTR that regulate its promoter activity in murine cells, but do not teach or even suggest that the MMTV promoter can be used to express a heterologous gene in a human cell. Furthermore, Lefebvre *et al.* do not teach a 0.6 Kb PstI fragment of MMTV, and thus, clearly do not teach a promoter element being present in such a promoter fragment of the MMTV (see, for example, Figure 2 of Lefebvre *et al.* which shows only one PstI restriction site).

Wilson *et al.* transfected corneal endothelial cells with the pMTV-D305 plasmid vector in which the SV40 large T antigen (SV40 LTAg) mRNA expression is positively regulated by the MMTV promoter. Wilson *et al.* teach that "[i]n the absence of dexamethasone, the proliferation of pMTV-D305-transfected cells was even slower, but cells continued to produce SV40 LTAg mRNA and protein" which indicated that "SV40 LTAg mRNA continued to be synthesized at significant levels in pMTV-D305-transfected cells in the absence of the inducer dexamethasone" (Wilson *et al.*, abstract, results). Wilson *et al.* do not teach or even suggest a 0.6 Kb PstI fragment of MMTV.

Similarly, Archer *et al.* do not teach or even suggest a 0.6 Kb PstI promoter fragment of MMTV. Archer *et al.* used the T47D (A1-2) cell line, a human mammary carcinoma-derived cell line engineered to constitutively express comparable levels of glucocorticoid and progesterone

receptors, to examine the transcriptional regulatory mechanisms of the receptors. As Archer *et al.* point out, the T47D (A1-2) cells also "possess *a stably integrated mouse mammary tumor virus (MMTV) luciferase reporter gene*" (Archer *et al.*, abstract, emphasis added). Clearly, Archer *et al.* also do not teach a retroviral vector or a DNA construct comprising a *heterologous* gene placed under transcriptional control of a 0.6 Kb PstI MMTV promoter fragment, wherein the MMTV promoter fragment directs expression of the heterologous gene in a cell when the vector is introduced into the cell.

The teachings of Dranoff *et al.* in view of Lefebvre *et al.*, Wilson *et al.*, Archer *et al.*, and Shao *et al.*, either alone or in combination, do not describe or even suggest a retroviral vector or a DNA construct comprising a heterologous gene placed under transcriptional control of a 0.6 Kb PstI MMTV promoter fragment, wherein the MMTV promoter fragment directs expression of the heterologous gene in a cell when the vector is introduced into the cell.

The teachings of Dranoff *et al.* in view of Lefebvre *et al.*, Wilson *et al.*, Archer *et al.*, and Shao *et al.* do not render obvious Applicants' claimed invention, particularly as amended.

CONCLUSION

In view of the above amendments and remarks, it is believed that all claims are in condition for allowance, and it is respectfully requested that the application be passed to issue. If the Examiner feels that a telephone conference would expedite prosecution of this case, the Examiner is invited to call the undersigned at (781) 861-6240.

Respectfully submitted,

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MARKED UP VERSION OF AMENDMENTS

Claim Amendments Under 37 C.F.R. § 1.121(c)(1)(ii)

1. (Three times amended) A retroviral vector comprising a heterologous gene placed under transcriptional control of [an] a 0.6 Kb PstI MMTV [regulatory sequence] promoter fragment, wherein the MMTV [regulatory sequence] promoter fragment directs expression of the heterologous gene in a cell when the vector is introduced into the cell.

13. (Twice amended) A retroviral provirus carrying a construct comprising a heterologous gene placed under transcriptional control of [an] a 0.6 Kb PstI MMTV [regulatory sequence] promoter fragment.

23. (Twice amended) A pharmaceutical composition comprising a DNA construct comprising a therapeutic gene placed under transcriptional control of [an] a 0.6 Kb PstI MMTV [regulatory sequence] promoter fragment, and a pharmaceutically acceptable carrier or diluent.

26. (Three times amended) A method for the expression of a heterologous gene in a human cell comprising introducing a retroviral vector comprising said gene under transcriptional control of [an] a 0.6 Kb PstI MMTV [regulatory sequence] promoter fragment into the human cell and maintaining the cell under conditions in which the gene is expressed in the human cell.

47. (Amended) The method according to claim [46] 41 wherein [the] a DNA construct [is] selected from the group consisting of: viral and plasmid vectors, is introduced into the human mammary cell.

74. (Amended) A retroviral vector comprising a heterologous gene placed under transcriptional control of a [rodent] 0.6 Kb PstI MMTV [regulatory sequence] promoter fragment, wherein the MMTV [regulatory sequence] promoter fragment directs expression of the heterologous gene in a human mammary cell when the vector is introduced into the cell.

79. (Amended) A retroviral provirus carrying a construct comprising a heterologous gene placed under transcriptional control of a [rodent] 0.6 Kb PstI MMTV [regulatory sequence] promoter fragment.

91. (Amended) A method for the expression of a heterologous gene in a human cell comprising introducing a retroviral vector comprising said gene under transcriptional control of a [rodent] 0.6 Kb PstI MMTV [regulatory sequence] promoter fragment into the human cell and maintaining the cell under conditions in which the gene is expressed in the human cell.

92. (Amended) A method for the treatment of human mammary carcinoma comprising administering to a human in need thereof a DNA construct comprising a therapeutic gene placed under transcriptional control of a [rodent] 0.6 Kb PstI MMTV [regulatory sequence] promoter fragment, wherein the therapeutic gene is expressed in human mammary carcinoma cells and the human mammary carcinoma is treated.